Real-time analysis of antibody binding interactions with immobilized *E. coli* O157:H7 cells using the BIAcore

The kinetic properties of antibody-antigen reaction and other interacting macromolecules can be analyzed in real-time using the surface plasmon resonance biosensor (BIAcore). The interactions of an antibody against *Escherischia coli* O157:H7 were studied using immobilized whole cells. The bacterial sensor surface was evaluated with anti-*E. coli* in a continuous flow system. Regeneration of the sensor surface with guanidine-HCl was more effective than with phosphoric acid and resulted in better binding reproducibility. The determined kinetic values, association and dissociation rate constants, can be used in the development of rapid immuno-techniques. This study also provides the basis to evaluate real-time interactions of macromolecules with immobilized cells.

Introduction

Recently, the BIAcore instrument, a surface plasmon resonance biosensor (SPR), has been utilized to determine the kinetic properties of the reaction between antibody and antigen and other interacting macromolecules based on a real-time biospecific interaction analysis (Karlsson *et al.*, 1991; Lofas *et al.*, 1991, Malmborg *et al.*, 1992; Malmquist, 1993; Fagerstram and O'Shannessy, 1993). This analysis allows a direct detection of the binding and dissociation rates of the reactions without using a signal generating label (Fagerstram, 1991; Fagerstram and O'Shannessy, 1991).

In this study, the BIAcore system was utilized to determine the feasibility of using immobilized cells as ligands to gain insight on the real-time interactions with cell surface specific antibodies; and to determine the association and dissociation rates and binding constants of an immunoglobulin (IgG) against *Escherichia coli* O157:H7 bacteria. The analysis of the binding and dissociation kinetics of an antibody and whole bacterial cell in suspension is usually tedious and impractical. The determined properties can then be utilized in the development of immuno-based biosensor techniques for rapid detection of *E. coli* O157:H7.

Materials and methods

The manufacturer's (Pharmacia Biosensor, Uppsala, Sweden) guidelines were followed for the preparation of the sensor surfaces, the binding and the regeneration of the injected antibody, the evaluation and the interpretation of the sensorgrams and kinetic evaluation.

Immobilization

Whole cells of *E. coli* O157:H7 irradiated with 12kGy of ^{137}Cs source were obtained from Gehring *et al.* (1996) and was prepared according to Thayer and Boyd (1993); and an affinity purified polyclonal goat IgG produced against heat-killed whole bacteria (KPL, Inc. Gaithersburg, Maryland) were utilized in this study. Stock suspensions of the bacteria at 3.33×10^5 and 3.33×10^7 cells per ml were prepared in 10 mM sodium acetate buffer, pH 4. The dextran surface of the flow cells on the sensor chip was activated with 30 µl of a mixture of equal volume of N-hydroxysuccinimide (NHS, 115 mg/ml) and N-ethyl-N′-(dimethylamino-propyl) carbodiimide (EDC, 750 mg/ml), followed by the injection of the bacteria. A 30 µl bacterial cell suspension containing 1×10^4 and 1×10^6 were immobilized on the surface of flow cells 1 and 2, respectively. The remaining activated dextran not immobilized with the cells was blocked with 15 µl ethanolamine. The reagents (EDC-NHS, bacteria, ethanolamine) were injected at 2 µl per min and Hepes buffered saline (HBS), pH 7.4, was utilized as the running buffer.

Binding and regeneration

Optimal binding pH, antibody concentration, binding period and flow rate were first determined. The optimum

conditions were then used for kinetic studies. With 50 μg anti-*E. coli* IgG/ml, the effects of pH on the binding were determined by using HBS buffers as the antibody diluent and running buffer. After selecting the optimum pH (pH 5), the binding interactions were monitored using serial dilutions of the antibody from 50 μg/ml to 6.25 μg/ml (333.3–41.7 nM). Other binding conditions were determined by observing the combined interactions of binding time from 10–30 min, flow rates of 2 or 3 μl per min and injection volume such that saturation of the sensor surface was achieved. The antibody bound sensor surface was regenerated by using 2 pulses (1 min each) of 6M guanidine-HCl, pH 1. (Brigham Burke and O'Shannessy, 1993; Fratamico *et al.*, 1996). In all these experiments the antibody was suspended in the same running buffers used as antibody diluent.

For regeneration and reproducibility studies, two flow cells in a new sensor chip were used to immobilize with 1×10^6 cells of *E. coli* O157:H7 as previously described. Anti-*E. coli* IgG (60 μl of 12.5 μg/ml) in HBS, pH 5 buffer was injected over the sensor surface in a flow rate of 2 μl/min. The immobilized *E. coli* sensor surfaces were regenerated by two pulses, 3 μl each of 6 M guanidine-HCl or 0.1 M phosphoric acid. The efficiency of the regeneration reagents were compared based on the completeness of the removal of the captured antibody and the ability of subsequent antibody binding. Separate flow cells were utilized for each regeneration reagent. The reproducibility of response units (RUs) generated by IgG binding and regeneration of the binding epitopes was determined in 6 replicate analysis.

Scanning electron microscopy (SEM)

Optical and electron microscopies were utilized to study the surface topography of the biospecific sensor surfaces on the flow cells. The sensor chip was imaged with reflected light using a stereomicroscope and digital images were collected. The sensor surfaces containing the immobilized cells were immersed in 1% glutaraldehyde fixative solution and stored at 4°C. The sensor chip was subsequently excised from the slide and washed in 0.1 M imidazole solution, dehydrated in 50% and absolute ethanol solutions, and critical point dried from liquid carbon dioxide. The chip was then mounted on an aluminum stub and sputter-coated with a thin layer of gold. The surface topography was revealed in the secondary electron imaging mode at 5kV accelerating voltage using JEOL USA Model JSM 840A scanning electron microscope. Digital images were collected at 1000× and 2,500×.

Results and discussions

Immobilization, binding and regeneration

Immobilization of 1×10^6 and 10^4 *E. coli* cells resulted in sensor surfaces with 90 and 14 RUs (response units) on flow cells 1 and 2, respectively. If higher binding surface is needed, the immobilization flow rate can be reduced or the immobilization time increased. However, the conditions described in this study was sufficient to meet our research objectives.

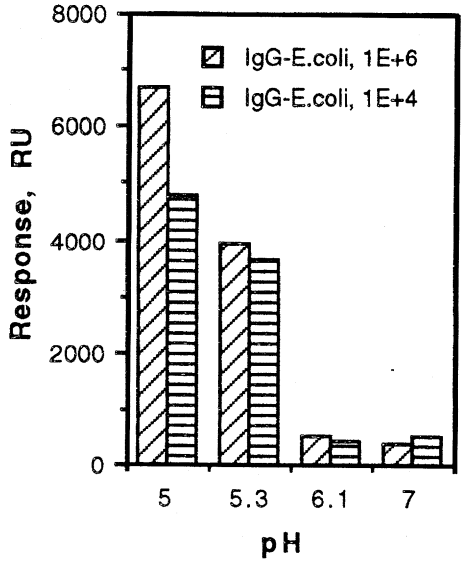

Figure 1 Effects of cell concentration and pH on the capture of *E. coli* O157:H7 IgG (50 μg/ml) by the immobilized bacterial cells. Sensor surfaces were immobilized with 1×10^6 cells and 1×10^4 cells.

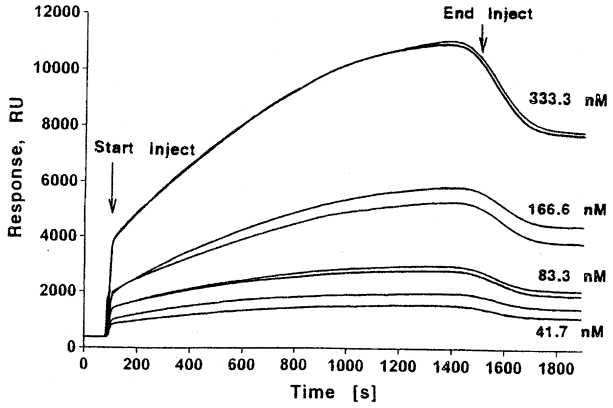

Figure 2 Overlay sensorgrams of the binding of anti-*E. coli* IgG by the sensor surface immobilized with 1×10^6 *E. coli* cells. Four concentrations of IgG (60 μl) were injected at a flow rate of 2 μl/min.

The antibody binding to the cell surface on flow cell 1 (1×10^6) and flow cell 2 (1×10^4) indicated that the bacterial surface in flow cell 1 captured more IgG (6700 RU) than in flow cell 2 (4800 RU) as shown in Figure 1. These responses correspond to 6.7 and 4.8 ng IgG per mm^2 of sensor surface (Karlsson *et al.*, 1991; Fagerstram and O'Shannessy, 1993). The pH effect on cell-antibody binding on these 2 sensor surfaces is also shown. Binding at pH 5 exhibited the highest response compared to pH 5.3, 6, and 7. The responses at pH 5 or 5.3 were 10 times greater than those at pH 6 or 7. Allowing the antibody to bind for 10–15 min was determined to be optimum as the response generally decreased when a 30 min binding period was used. In current studies and in other systems, a higher response was observed for binding with a flow rate of 2 µl per min compared to 3 µl or 5 µl per min (Fratamico *et al.*, 1996).

For kinetic analysis, a 30 min binding was used to achieve saturation of the sensor surface on flow cell 1 (1×10^6) . The binding responses generated by the different concentrations of antibody are shown in an overlay sensorgram (Figure 2). The apparent association (k_a) and dissociation rate (k_d) constants were determined from these sensorgrams and are shown in Table 1.

The apparent k_d values were similar in the two lower and higher concentrations of the injected antibody. The sensorgrams also show a large excess of IgG in the 166 and 333 nM concentrations compared to the mass of the captured IgG. The affinity constants (K) calculated from the ratio of the association rate constant to the dissociation rate constant showed the apparent K values decreased with the higher IgG concentration. The observed changes in these kinetic and thermodynamic constants indicated the presence of multiple binding processes between the cell surface and the applied polyclonal antibody. Thus, the faster binding rate and stronger binding processes dominated the determined data when the applied antibody concentration was low. As the antibody concentration increased, the slower rate and weaker binding processes became more significant to the overall measurements. Consequently, the apparent rate constants and binding strength decreased as the applied antibody concentration increased.

Table 1 Apparent kinetic values of the biomolecular interaction of polyclonal *E. coli* O157:H7 antibody with immobilized whole cells (1×10^6) determined as averages of duplicate analysis

nM IgG	*Association Rate Constant* k_a ($M^{-1}s^{-1}$)	*Dissociation Rate Constant* k_d (s^{-1})	*Affinity Constant, K =* k_a/k_d, (M^{-1})
41.7	1.35×10^4	2.05×10^{-5}	5.30×10^8
83.3	1.20×10^4	6.20×10^{-5}	1.94×10^8
166.6	6.00×10^3	8.25×10^{-5}	7.28×10^7
333.3	1.93×10^3	8.63×10^{-5}	2.24×10^7

There was an apparent reduction (1–22%) in binding response in the second analysis of the duplicate samples. In contrast, there was a gradual increase in the absolute RU of the baseline of the cell-sensor surface in 12 experiments. This decrease in antibody binding can be attributed to saturation of the binding sites by previous analysis. Apparently, the regeneration step did not completely remove the antibody or the cell surface was altered by the analytical processes. Results from 6 replicate analysis (Figure 3) of IgG binding and guanidine-HCl regeneration showed a 27% increase in RU (3016). However, the bound IgG had only an increase of 54 RU which is 1/3 of the sample standard deviation, 146 RU. In contrast, the IgG binding and phosphoric acid regeneration cycle resulted in a 90% (9880 RU) increase in baseline and gradually inhibited IgG binding with a reduction of 1910 RU, a 35% decrease from the first replicate analysis. This decrease in IgG bound RU was 3 times the sample standard deviation of 639 RU. These results showed that 6M guanidine-HCl was more effective than phosphoric acid in regenerating the binding epitopes on the bacterial surface.

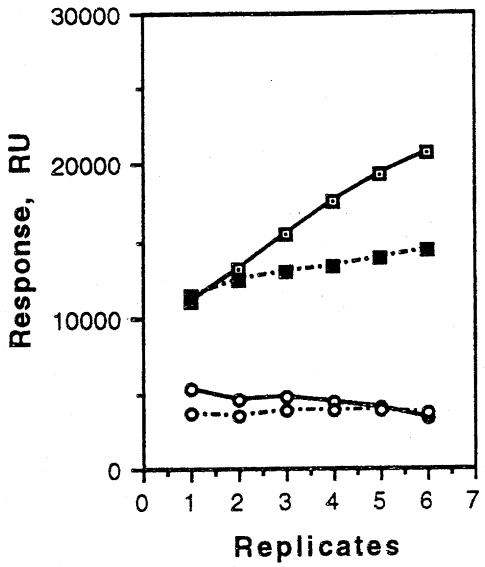

Figure 3 Regeneration effects of 6M guanidine-HCl (·—·) and 0.1 M phosphoric acid (–) in 6 replicate analysis. Baseline Absolute RU (□), Relative RU of captured *E. coli* IgG (○).

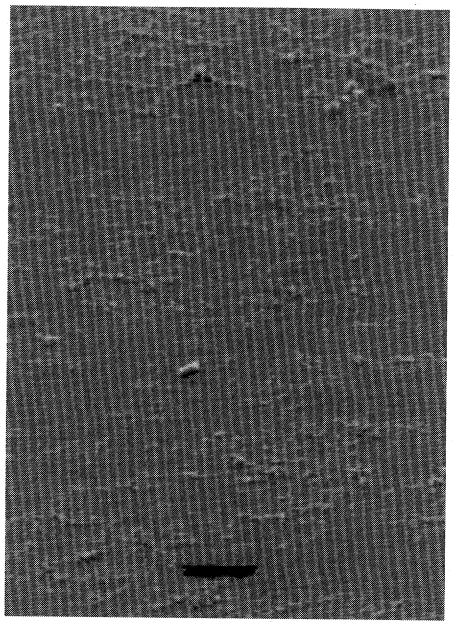

Figure 4 SEM micrograph (scale bar = 5 μm) of the sensor surface immobilized with 1×10^6 cells of *E. coli* O157:H7 and regenerated with guanidine/HCl.

SEM and optical microscopy

Scanning electron microscopic (SEM) examination of freshly immobilized cells, at a magnification of 2500×, revealed that the cell distribution is discrete and scattered. SEM examination of the regenerated cell surfaces indicated the change in cellular structure from the rod-shaped cells (on unregenerated flowcell) to a more continuous ridges of surface coating on the regenerated sensor chip (Figure 4). These surfaces were in contrast to a smooth surface of the unmodified flowcells. These results also suggest that the cell surface coating remained attached to the sensor chip while the cell walls were lost. Optical analysis of the regenerated immobilized surface showed binding to an FITC-labeled anti-*E. coli* antibody and this indicated that the binding components remained attached to the sensor surface shown by the fluorescence signals. The intensity of the fluorescent signals were higher in the regenerated cell surface than in the intact cells suggesting availability of more cell surface antigens or binding epitopes. These results demonstrate the immobilization of cell surface antigens from intact cells for the binding studies.

Conclusions

Real-time biospecific interaction analysis of an antibody and immobilized whole bacterial cell generated the kinetic information describing antibody binding to the cell surface. The antibody produced against heat-killed bacteria, also bound with the surfaces of the irradiated bacteria used as sensor surface. This study demonstrates that the binding epitopes of the cell surfaces were identical in both treatments. The association and dissociation rate and binding constants were determined in pH 5 using varying the antibody concentrations. Guanidine-HCl is more effective than phosphoric acid in regenerating the bacterial surface bound with IgG. However, the regeneration process appeared to change the bacterial surface structural topography but retained the antibody binding epitopes. The amount of bound IgG after the regeneration cycle remained the same in 6 replicate analysis. The information obtained from these studies should be useful in guiding further real-time studies and in gaining insight on interactions between macromolecules and immobilized whole cells.

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